

Analysis of Genes Expressed in Response to Cold Temperatures Under Different Photoperiods in Peach Bark

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The behavior of all cells and their response to environmental change is governed by the induction and repression of various groups of genes. Global approaches to identifying genes that are modulated in response to stress have been successfully applied to several plant and animal systems. However, certain limitations restrict the degree to which each approach is successful in documenting true differences in expression. For example, microarray analysis is restricted to previously isolated genes and does not allow identification of unique, undiscovered genes that might be crucial to the response being studied. EST library approaches overcome this problem in part, but are labor-intensive and tend to be somewhat biased for genes that are moderately-to-highly abundant. One approach that overcomes these limitations is the synthesis of gene libraries by subtractive hybridization and cloning. By subtracting cDNAs synthesized from mRNAs expressed in one state from cDNAs derived from mRNAs expressed in another state one can obtain sequences that are modulated when comparing the two mRNA populations side-by-side because sequences common to both populations are removed by hybridization. By varying which cDNA serves as the driver of the hybridization reaction and which cDNA serves as the tester, one can obtain both up-regulated (forward subtraction) and down-regulated (reverse subtraction) sequences in response to a defined set of experimental conditions. In an effort to profile gene expression at different temperatures under different photoperiods, we have created subtracted libraries from peach (*Prunus persica*) bark tissues sampled from trees maintained at 5° and 25° under a short day (SD) photoperiod or exposed to a night break (NB) interruption during the dark period of the SD cycle. Differentially expressed sequences enriched by performing forward and reverse subtractions using various combinations of temperature and photoperiod treatments were cloned, sequenced, and identified by BLAST analysis. The results have been analyzed relative to known or predicted functions of the gene products identified and their association with the various experimental manipulations.